

FORM PTO-1339
(REV. 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

5452-US

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

09/889530

INTERNATIONAL APPLICATION NO.
PCT/US00/02026INTERNATIONAL FILING DATE
26 JAN 2000 (26.01.00)PRIORITY DATE CLAIMED
26 JAN 1999 (26.01.99)

TITLE OF INVENTION

TREATMENT OF NEOPLASMS WITH YIJUNGAMYCINS

APPLICANT(S) FOR DO/EO/US

SKITBO, EDWARD B.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☐ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ has been communicated by the International Bureau.
 - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☐ is attached hereto.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☒ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information: CHANGE OF CORRESPONDENCE ADDRESS

U.S. APPLICATION NO. **09/889530**INTERNATIONAL APPLICATION NO.
PCT/US00/02026ATTORNEY'S DOCKET NUMBER
5452-US21. ☒ The following fees are submitted:**BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):**Neither international preliminary examination fee (37 CFR 1.482)
nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO
and International Search Report not prepared by the EPO or JPO **\$1000.00**International preliminary examination fee (37 CFR 1.482) not paid to
USPTO but International Search Report prepared by the EPO or JPO **\$860.00**International preliminary examination fee (37 CFR 1.482) not paid to USPTO
but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **\$710.00**International preliminary examination fee (37 CFR 1.482) paid to USPTO
but all claims did not satisfy provisions of PCT Article 33(1)-(4) **\$690.00**International preliminary examination fee (37 CFR 1.482) paid to USPTO
and all claims satisfied provisions of PCT Article 33(1)-(4) **\$100.00****ENTER APPROPRIATE BASIC FEE AMOUNT =****CALCULATIONS PTO USE ONLY**

\$ 100.00

Surcharge of \$130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30
months from the earliest claimed priority date (37 CFR 1.492(e)).

\$

CLAIMS NUMBER FILED NUMBER EXTRA RATE \$Total claims 10 - 20 = 0 x **\$18.00** \$Independent claims 3 - 3 = 0 x **\$80.00** \$MULTIPLE DEPENDENT CLAIM(S) (if applicable) + **\$270.00** \$**TOTAL OF ABOVE CALCULATIONS =** \$ 100.00☒ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above
are reduced by 1/2. + \$ 50.00**SUBTOTAL =** \$ 50.00Processing fee of \$130.00 for furnishing the English translation later than ☐ 20 ☐ 30
months from the earliest claimed priority date (37 CFR 1.492(f)). \$**TOTAL NATIONAL FEE =** \$Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be
accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **\$40.00** per property + \$**TOTAL FEES ENCLOSED =** \$ 50.00Amount to be
refunded: \$

charged: \$

- a. ☒ A check in the amount of \$ 50.00 to cover the above fees is enclosed.
- b. ☐ Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees.
A duplicate copy of this sheet is enclosed.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any
overpayment to Deposit Account No. 135100. A duplicate copy of this sheet is enclosed.
- d. ☐ Fees are to be charged to a credit card. **WARNING: Information on this form may become public. Credit card
information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR
1.137 (a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

RICHARD R. MYBECK
NAME17,886
REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Edward B. Skibo

Attorney Docket: 5452-US

Serial No:

Group Art Unit:

Filed:

Examiner:

For: Treatment of Neoplasms with Yujungamycins

PRELIMINARY AMENDMENT

To: Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please insert the following in the Specification.

Page 1, line 1,

--This Application is based on United States Provisional Application S/N
60/117,199 filed January 26, 1999 which was filed as PCT Serial Number
PCT/US00/02026 on International Filing Date January 26, 2000 and assigned
International Publication Number WO 00/43005, which was published on July 27, 2000.-

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Respectfully submitted,



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Reg. No. 17,886

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CERTIFICATE OF EXPRESS MAIL

EL742013980US

Express Mail Number

18 July, 2001

Date of Deposit

I hereby certify that this correspondence is being deposited with the United States Postal Service
"Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated and is addressed to:
Assistant Commissioner for Patents, Box PCT, Washington, D.C. 20231.

18 July, 2001

Date


Richard R. Mybeck

Rec'd PCT/PTO 1 8 JUL 2001

1

Treatment Of Neoplasms With The Yujungamycins

The present invention relates to compounds derived from the pyrrolo[1,2- α]benzimidazole ring system and the discovery of their unique and unexpected antineoplastic activity, particularly against central nervous system, ovarian, and lung cancers.

Financial assistance for this project was provided by U.S. Government through the National Science Foundation under Grant Number CHE-9522640 and through the National Institutes of Health under Grant Number CA 73758; and the United States Government may own certain rights to this invention.

Background Of The Invention

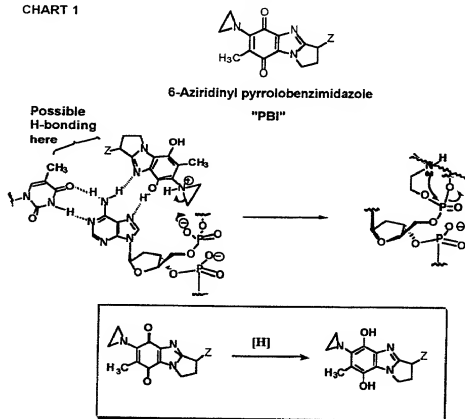
Work in this laboratory has been involved with the design of a new class of DNA cleaving agent based on the pyrrolo[1,2- α]benzimidazole (PBI) ring system for some time (Skibo, E.B. and Schulz, W.G. Pyrrolo[1,2- α]benzimidazole-Based Aziridinyl Quinones. A New Class of DNA Cleaving Agent Exhibiting G and A Base Specificity. *J.Med.Chem.* 36: pp3050-3055, 1993; *op. cit.*, Schulz, Nieman and Skibo, 1995.) These agents were designed to alkylate the phosphate backbone upon reduction to create a hydrolytically-labile phosphotriester, Chart 1. While in the reduced form, these agents can hydrogen-bond to the major groove and thereby recognize base pairs. (Skibo, E.S., Gordon, S., Bess, L., Boruah, R. and Heileman, J. Studies of Pyrrolo[1,2- α]benzimidazole Quinone DT-Diaphorase Substrate Activity, Topoisomerase II Inhibition Activity, and DNA Reductive Alkylation. *J.Med.Chem.* 40:pp1327-1339, (1997).) The reducing enzyme DT -diaphorase can activate antitumor agents by two-electron reduction in both normal and cancerous tissues, (*op. cit.* Riley and Workman, 1992; Marin, A., deCeran, A.L., Hamilton, E., et al. DT-diaphorase and cytochrome B-5 reductase in human lung and breast tumors. *Br.J.Cancer Vol 76, Iss 7*, pp923-929, 1997) as illustrated in the inset of Chart 1. Depending on the organ and species source, the substrate and inhibition properties of the enzyme can vary widely (*op. cit.*, Rauth, Goldberg and Misra, 1997; Wu *etc.* 1997). This inventor maintains that since the structure of DT-

diaphorase varies by organ system, the enzyme could also vary by the organ system cancer. Thus cancers originating from these organs (lung, renal, ovarian, etc.) will possess slightly different DT-diaphorase, assuming some degree of differentiation. The PBIs possess variable cytotoxicity in cell lines and a corresponding variable activity *in vivo* models. Therefore this inventor has conducted the synthesis and cytotoxicity/*in vivo* screens of both known and new PBIs.

The previously reported structure corresponding to Yujungamycin A (*op. cit.* Skibo, Gordon et al., (1997) possessed low cytotoxicity in cell lines. However, the present application shows that Yujungamycin A is unexpectedly active *in vivo*.

- 10 Therefore, Yujungamycin B and C were prepared to exploit this unexpected finding.

CHART 1



Some bulky substituents and stereochemistry (*op. cit.* Skibo *etc.* 1997) are known to influence the substrate specificity of DT- diaphorase. In the present invention both the stereochemistry and the hydrogen bonding capabilities represented by the enantiomers of yjungamycins A, B, C. were employed to influence DT-diaphorase substrate specificity.

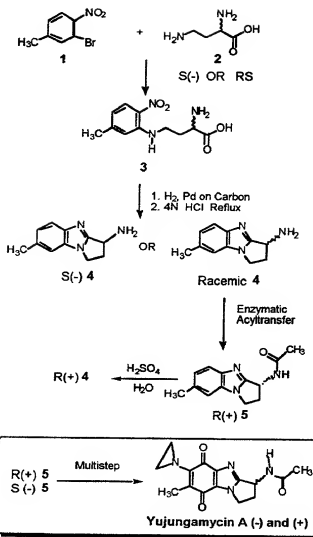
Synthesis

The preparation of the yjungamycins is outlined in Schemes 1 and 2.

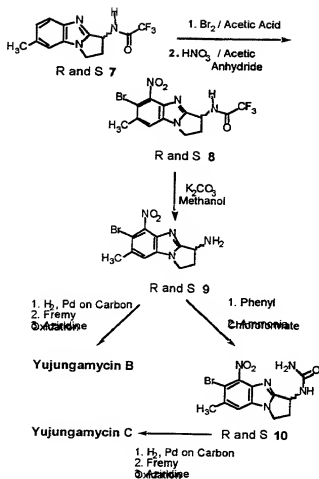
Previous synthetic studies led to the preparation of racemic **4** employing 1 and either racemic or S(-)**3**. (*op cit* Skibo *etc.* 1997; Skibo, E.B., Islam, I., Schulz, W.G., Zhou, R., Bess, L. and Boruah, R. The Organic Chemistry of the Pyrrolo[1,2-a]benzimidazole Antitumor Agents. An Example of Rational Drug Design. Synlett pp297-309, (1996).; Skibo, E.B. Pyrrolobenzimidazoles in cancer treatment. Expert.Opin.Ther.Patents. Vol 8, Iss 6:pp673-701, (1998)). Acetylation of S(-)**4** to S(-)**5** was followed by conversion to yjungamycin A(-), which is the S enantiomer, Scheme 1. The previously unknown R(+) enantiomer was prepared from racemic **4** employing an acyltransferase (ALTUS 20 CLEC catalyst), which afforded R(+)**5** as the major product. Altus Inc. provides a kit of 40 acyltransferases to screen for enantiomeric excesses, only the mentioned acyltransferase gave a suitable result. Conversion of R(+)**5** to the R(+) form of yjungamycin A was carried out as previously described (*op. cit.*, Skibo *etc.* 1997).

In order to prepare the R(+) or the S(-) forms of yjungamycin B and C, the R(+) or S(-) forms of **4** were trifluoroacetylated to afford **7**, Scheme 2. The trifluoroacetyl group was easily removed after the bromination and nitration reactions to afford the common intermediate **9**. Yjungamycin B was prepared from **9** by reduction, Fremy oxidation, and finally aziridination as previously described for the PBIs. (*op. cit.*, Skibo *etc.* 1996) The same procedure was employed to convert the 3-carbamido derivative **10** to yjungamycin C.

SCHEME 1



SCHEME 2



Antitumor Activity

In vivo screening results of Yujungamycin A, employing the new hollow fiber assay, are discussed in conjunction with Table I and Figures 1 and 2.

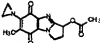
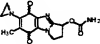
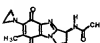
Hollow fiber assays are carried out in the following way. Human tumor cells are cultivated in polyvinylidene fluoride hollow fibers, and a sample of each cell line is implanted into each of two physiological compartments (intraperitoneally and subcutaneously). Mice are treated with either a high or a low dose using a QD x 4 schedule (four daily treatments) administered intraperitoneally. Altogether, twelve cell lines are studied resulting in 48 possible test combinations (12 cell lines x 2 sites x 2 doses). A score of two is given to each test in which there is a % T/C of 50 or less (tumor mass 50% or less than the control. Thus the highest possible score is a 96, but the typical score is only 5 and the highest score achieved is a 64. Typically

the score is broken down into a intraperitoneal (IP) and a subcutaneous (SC) score. A good SC score (≥ 8) indicates that the drug is able to get to the tumor site(subcutaneous) from a distant site(intraperitoneal) of injection. Both PBI-A and PBI-B shown in Table 1 are active in the hollow fiber assay and are active against melanoma, NSC lung cancer, breast cancer, and ovarian cancer. These compounds are covered by two patents. (Skibo, E.B. and Islam, I. Synthesis and Elucidation of Azamitosene and Iminoazamitosene. *U.S. Patent 5,015,742* 1991; Skibo, E.B., Islam, I. and Alberts, D.S., Antineoplastic Agents and Methods of Using the Same. *U.S. Patent 5,246,955*, 1993).

10 The three substituent of the PBI is crucial of cytotoxic activity, but is not required for the actual reductive alkylation process(requires the aziridinylquinone). The 3-unsubstituted PBI and the 3-hydroxy PBI are devoid of activity while the lipophilic 3- propanoate derivative showed animal toxicity (100% drug deaths) at doses as low as 5 mg/kg without any significant antitumor activity. The acetate and the carbamate, PBI-A and PBI-B respectively, appear to have a suitable balance of polarity and lipophilicity in the 3- substituent for antitumor activity. These findings prompted the investigation of the yujungamycins, all of which bear a nitrogen bound to the 3-position. The resulting acetamido, amino, or carbamido substituents are more stable to hydrolysis than the corresponding oxygen derivatives

20 (PBI-A and PBI-B). Therefore it was postulated that the yujungamycins would be more cytotoxic than the PBIs. The interaction of these substituents with DT-diaphorase would contribute to the selective cytotoxicity of these agents. Indeed, the data in Table 1 shows that yujungamycin A(-) did obtain a higher score than the PBIs. The high subcutaneous score of 14 indicated that this agent can kill cancer cells at a location distant from the site of injection.

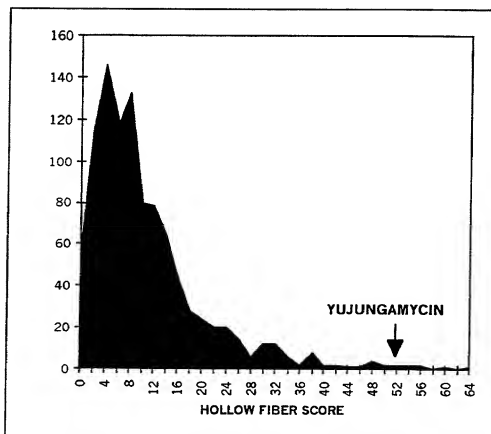
Table 1

	Intraperitoneal Score	Subcutaneous Score	Total Score	
	40	8	48	Cancer cell line types sensitive to the PBIs: Melanoma Non-Small Cell Lung Cancer Breast Cancer Ovarian Cancer
PBI-A				
	24	6	30	
PBI-B				
	38	14	52	Cancer cell line types sensitive to A(+): Non-Small Cell Lung Cancer CNS Cancer Ovarian Cancer
Yujungamycin A (-)				

The National Cancer Institute has tested hundreds of new compounds, either synthetic or natural products, in the hollow fiber screen. (Hollingshead, M., Plowman, J., Alley, M., Mayo, J. and Sausville, E. Relevance of Tumor Models in Anticancer Drug Development. In: *Contributions to Oncology*, Germany: Krager Verlag, 1998). These compounds are active *in vitro* and therefore chosen for the *in vivo* hollow fiber assay. Figure 1 shows the position of yujungamycin A(-) among the compounds screened at the National Cancer Institute. The typical antitumor agent only has a hollow fiber score of ~5, while yujungamycin A(-) scores at 52 making it one of the most active compounds studied by the National Cancer

10 Institute.

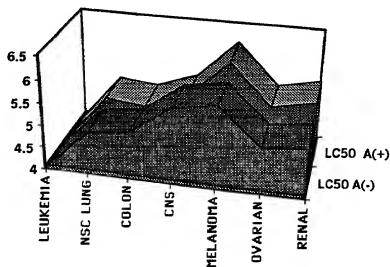
Figure 1. Plot of Number of compounds studied at the National Cancer Institute versus their hollow fiber score.



The $-\log LC_{50}$ data in Figure 2 confirmed the prediction that selective cytotoxicity would be exhibited by the yujungamycins. Thus, yujungamycin A(+) shows substantially higher cytotoxicity against melanoma than the (-) enantiomer.

- 10 However, the magnitude of the cytotoxicity (highest $-\log LC_{50}$ only 6) is much less than the previously studied PBIs. (*op. cit.*, Skibo, 1998). The extraordinary *in vivo* activity exhibited by yujungamycin A(-) was entirely unexpected. If cell line data is an indication of relative activity, then the more active A(+) enantiomer should be even more effective *in vivo*.

Figure 2. Plots of average $-\log LC_{50}$ values versus the cancer type for yujungamycin A(+) and A(-). Each $-\log LC_{50}$ value is the average of up to eight values.



The results of human melanoma tumor syngraft studies with R(+) and S(-) Yujungamycin A, Figure 3, failed to show much difference in activity between the enantiomers. Although it should be pointed out that the enantiomeric differences apparent in Figure 2 reflect the average of several cell lines for each cancer category. Perhaps the enantiomeric differences will become apparent when more syngraft studies are carried out. However, the activity of S(-)-Yujungamycin A in the syngraft assay distinguishes it from other compounds with high hollow fiber assay scores. Close inspection of Figure 1 shows that there are a few compounds with higher scores than S(-)-Yujungamycin A, the highest score being 64 for a natural product. Many of these compounds are too toxic and do not show activity *in vivo*.

Besides studying the influence of stereochemistry, the hydrogen bonding capabilities of the 3-substituent was also varied. The properties of the 3-substituent are crucial for antitumor activity with low toxicity. Extremes include the 3-hydroxy derivative, which is nearly devoid of cytotoxicity, and the 3-propanoate, which is

very cytotoxic but too toxic for *in vivo* use. Figure 1 shows the increasing activity in the hollow fiber assay as the 3-substituent is changed from carbamate (PBI-B), to acetate (PBI-A), and finally to 3-acetamido (Yujungamycin A). When the 3-hydroxy group is changed to 3-amino (Yujungamycin B), there is ~million-fold change in cytotoxicity. This finding suggests that a basic group in the 3-position is required for cytotoxicity, since the large difference in cytotoxicity seems to reflect the relative pKa values of a protonated amine versus a protonated alcohol. Figure 4 contrasts the relative cytotoxicities of the S(-) forms of Yujungamycins A, B, C against several types of cancer. The high potency of Yujungamycin B, compared to the others, illustrates the importance of 3-substituent basicity on cytotoxicity. Preliminary studies indicate the 3-amino substituent greatly enhances the alkylation of DNA, perhaps explaining the high cytotoxicity.

Figure 3. Results of S(-)- and R(+)-Yujungamycin A in the B-16 melanoma model in C57/bl mice. These compounds were studied at two dose levels: 1 and 3 mg/Kg IP on days 1, 5, and 9 after tumor implantation into the front flank muscle. Shown in the bar graph below is the area under the tumor versus time curve plotted against dose for each enantiomer. Note that there are no apparent differences between the enantiomers in these data.

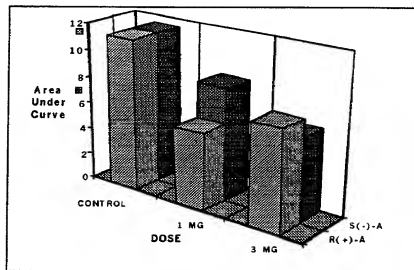
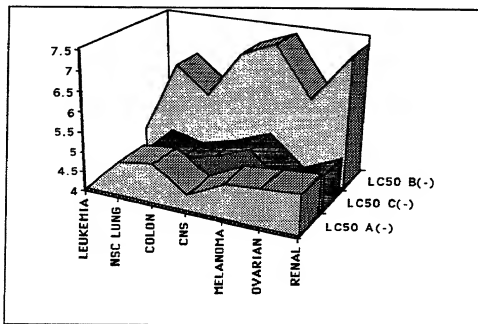


Figure 4. Plots of $-\log LC_{50}$ versus cancer type for S(-)-1, 2, 3. Each cancer type represents the average of six to eight different cancer cell lines. The 3-amino derivative 2 shows high cytotoxicity against non-small-cell lung, central nervous system, melanoma, ovarian, and renal cancers.



Experimental Section

(R) - (+) -3 - Trifluoroacetamido- 2, 3 - dihydro- 7-methyl - 1H - pyrrolo[1,2-a] benzimidazole (R(+))7) was prepared from racemic 4 by the following three-step process.

To 543 mg (2.9 mmol) of racemic 4 in a 250 mL round bottom flask was added 310 mg of ALTUS 20 CLEC catalyst along with 104 mL of ethyl acetate. The resulting mixture was stirred vigorously for 30 min and solids were filtered off and washed 3x with 10 mL portions of $CHCl_3$. The extraction solvent was removed under reduced pressure to afford a residue, which was placed on a silica gel column employing 3% methanol in chloroform as the eluent. Pure R(+) 5 eluted from the column as the first band. The product was recrystallized from ethyl acetate / hexane: 244 mg (37%) yield; mp 219-220; TLC (chloroform/methanol

[90:10] $R_f = 0.38$; IR (KBr pellet) 3245, 3068, 2990, 1635, 1560, 1420 cm^{-1} ; ^1H NMR (CDCl_3) δ 7.75 (1H, s, acetamido proton) 7.47 (1H, d, $J = 8.4$ Hz, 5-aromatic proton), 7.02 (1H, d, $J = 8.4$ Hz, 6-aromatic proton), 6.88 (1H, s, 8-aromatic proton), 5.41 (1H, m, 3-methine proton), 4.0-3.8 (2H, m, 1-methylene protons), 3.25-3.15 (1H, m, 2-methylene proton), 2.55-2.39 (1H, m, 2-methylene proton) 2.43 (3H, s, methyl), 2.09 (3H, s, methyl); mass spectrum (EI) m/z : 229 (M^+), 186 ($\text{M}^+ - \text{COCH}_3$), 171, 158, 145, 133, 116, 104. Rotations: (R)-(+)[α] $_D^{25} = +104.4^\circ$ ($C = 0.41$, MeOH).

10 The deacetylation of R-(+) **5** was carried out by dissolving 210mg (0.877 mmol) in 12 mL of 1.2N hydrochloric acid and refluxing the solution for 9h. The solvent was removed under vacuum to afford a white solid, which was dissolved in water and then buffered to pH 7 with saturated sodium bicarbonate solution. The neutralized solution was extracted 5x with 30 mL portions of chloroform and the extracts dried over sodium sulfate. Concentration of the dried extracts and recrystallization from chloroform / hexane afforded 155 mg (95%) of R-(+) **4** as a white solid. Rotations: R-(+) **4** [α] $_D^{25} = +20.9^\circ$ ($C = 1.1$, MeOH) and S-(-) **4** [α] $_D^{25} = -20.7^\circ$ ($C = 0.2$, MeOH).

20 Trifluoroacetylation of R-(+) **4** was carried out by dissolving 155 mg (0.83 mmol) in 3 mL of trifluoroacetic acid followed by addition of 3 mL of trifluoroacetic anhydride. The reaction mixture was stirred for 20 min at room temperature and then poured into 150 mL of 0.1M pH 7.0 phosphate buffer. The resulting solution was extracted 3x with 20 mL portions of ethyl acetate and the extracts were washed 2x with 10 mL portions of saturated sodium bicarbonate and then dried over sodium sulfate. The extracts were concentrated to a residue, which was recrystallized from ethyl acetate / hexane to afford 152 mg (65%) yield of R-(+) **7**. Rotation: (R)-(+)[α] $_D^{25} = +101.2^\circ$ ($C = 0.55$, MeOH). Anal. ($\text{C}_{13}\text{H}_{12}\text{F}_3\text{N}_3\text{O}$) C , H , O .

(S) - (-) - 3 - trifluoroacetamido- 2, 3 - dihydro- 7-methyl - 1H - pyrrolo[1,2-a] benzimidazole (S(-)7). To a mixture of 683 mg (3.05 mmol) of S(-) 4 was dissolved in 20 mL trifluoroacetic acid. To this mixture was added 20 mL trifluoroacetic anhydride and the reaction was stirred for 25 min at room temperature. The completed reaction was poured into 700 mL of 0.1 M pH 7.0 phosphate buffer. This solution was extracted 4 x with 40 mL portions of ethyl acetate and then the extracts were washed 3 x with 20 mL portions of saturated aqueous bicarbonate solution and dried over Na₂SO₄. The solvent was removed under reduced pressure and the product recrystallized from ethyl acetate / hexane: 546 mg (63%) yield; mp 227-228°C; TLC (chloroform/methanol [90:10]) *R_f* = 0.68; IR (KBr pellet) 3178, 2987, 2912, 2858, 1724, 1572, 1523, 1213, 1186, 1153 cm⁻¹; ¹H NMR (CDCl₃) δ 9.75 (1H, bs, -NH), 7.41 (1H, d, *J* = 8.1 Hz, 5 - aromatic proton) 7.02 (1H, d, *J* = 8.1 Hz, 6-aromatic proton), 6.79 (1H, s, 8-aromatic proton), 5.43 (1H, q, *J* = 6.3 Hz, 3-methine proton), 4.08-3.89 (2H, m, 1-methylene proton), 3.31 - 3.89 (1H, m, 2-methylene proton), 2.69 - 2.58 (1H, m, 2-methylene proton), 2.41 (3H, s, 2-methyl protons); mass spectrum(EI mode) *m/z* 283(M⁺), 186 (M⁺ - CF₃CO), 121, 131, 105. Rotation: S(-) 7 [α]_D²⁵ = -108.7° (C = 0.42, MeOH). Anal. (C₁₃H₁₂F₃N₃O)C, H, O.

6-Bromo - 3 - trifluoroacetamido- 2, 3 - dihydro- 7-methyl - 5 - nitro - 1H - pyrrolo[1,2-a]benzimidazole (8) was prepared by the following two - step procedure starting with 7:

To a solution of 500 mg (1.76 mmol) of 7 in 25 mL of acetic acid was added 0.25 mL of a solution of bromine 0.8 M in acetic acid. The resulting solution was stirred at room temperature for 20 min and then quenched by dilution with 500 mL of 0.1 M pH 7.0 phosphate buffer. The resulting mixture was extracted 3x with 80 mL portions of ethyl acetate and then the extracts were washed 2x with 80 mL portions of saturated aqueous sodium bicarbonate solution. The extracts were dried over Na₂SO₄ and the solvent removed under reduced pressure to afford the bromo derivative which was recrystallized from ethyl acetate/hexane: 546 mg(85%) yield: m.p. 215-217°C; TLC (chloroform / methanol[90:10]) *R_f* = 0.50, IR (KBr

pellet) 3178, 2984, 2859, 1730, 1570, 1522, 1445, 1221, 1186, 1148 cm^{-1} ;
 $^1\text{H NMR}$ (CDCl_3) δ 9.9 (1H, bs, amide NH), 7.61 (1H, s, 5-aromatic proton), 6.83
 (1H, s, 8-aromatic proton), 5.48-5.40 (1H, m, 3-methine proton), 4.17-3.95 (2H,
 m, 1-methylene protons), 3.33-3.26 (1H, m, 2-methylene proton), 2.77-2.65 (1H m,
 2-methylene proton), 2.42 (3H, s, methyl); MS (EI) m/z 363 & 361 (M^+ , ^{79}Br &
 ^{81}Br), 264 & 266 ($\text{M}^+ - \text{COCF}_3$), 223, 169, 130, 90. Rotations: (R) - (+) $[\alpha]_{\text{D}}^{25} =$
 $+ 92.6^\circ$ (C=0.47, MeOH); (S) - (-) $[\alpha]_{\text{D}}^{25} = - 97.8^\circ$ (C=0.18, MeOH). Anal.
 ($\text{C}_{13}\text{H}_{11}\text{BrF}_3\text{N}_3\text{O}$) C, H, N.

- To 12 mL fuming nitric acid, cooled to 0°C , was slowly added 500 mg (1.4
 10 mmol) of the bromo derivative with stirring. To the resulting solution was added
 1.14 mL of acetic anhydride with continued stirring and cooling. After stirring the
 reaction mixture at 0°C for 5 min., the reaction mixture was allowed to come to
 room temperature and then stirred for 1.5 hr. The completed reaction was poured
 into 450 mL of ice water and the mixture buffered to pH 7 with NaHCO_3 . The
 1 product was extracted 3 x with 50 mL portions of ethyl acetate. The extracts were
 then dried over Na_2SO_4 and solvent removed to afford a yellow residue.
 Recrystallization from a minimum amount of ethyl acetate, facilitated by the
 addition of hexane, afforded **8** as light yellow crystals: 466mg (89 %) yield; mp
 245-247 $^\circ\text{C}$; TLC (chloroform/methanol [90:10]) R_f =0.60; IR (KBr pellet) 3246,
 20 3044, 1741, 1657, 1570, 1538, 1370, 1229, 1159 cm^{-1} , $^1\text{H NMR}$ (CDCl_3) δ 10.21
 (1H, s, amide NH), 7.38 (1H, s, 8-aromatic proton), 4.75-4.70 (1H, m, 3-methine
 proton), 4.52- 4.42 (1H, m, 1-methylene proton), 4.20-4.12 (1H, m, 1-methylene
 proton), 3.50-3.40 (1H, m, 2-methylene proton), 2.95-2.85 (1H, m, 2-methylene
 proton), 2.58 (3H, s, methyl); MS (EI Mode), m/z 406 (M^+), 361 ($\text{M}^+ - \text{NO}_2$),
 309, 293, 280, 263, 189. Rotations: R(+) $[\alpha]_{\text{D}}^{25} = +70.12^\circ$ (C = 0.42, MeOH); S(-)
 $[\alpha]_{\text{D}}^{25} = - 64.0^\circ$ (C = 0.39, MeOH).
 Anal. ($\text{C}_{13}\text{H}_{10}\text{BrF}_3\text{N}_4\text{O}_3$) C, H, N.

3-Amino - 6 - bromo - 2,3 - dihydro -7- methyl - 5 - nitro - 1H - pyrrolo [1,2-*a*] benzimidazole(9). To a solution of 45 mL of methanol cooled to -70°C in an isopropanol/dry ice bath was bubbled gaseous ammonia until the volume of liquid doubled. To the resulting solution was added 300 mg (0.737 mmol) of **8** and the mixture was then removed from the dry ice bath and stirred for 48h at room temperature. The solvent was evaporated and the residue was recrystallized from chloroform/hexane to afford light brown crystals: 151 mg (66%) yield; mp 145-148°C; TLC (chloroform/methanol [80:20]) R_f = .22; IR (KBr pellet) 3389, 2924, 1532, 1458, 1381, 1294, 878 cm^{-1} ; ^1H NMR (CDCl_3) δ 7.36 (2H, s, aromatic proton), 4.58 (1H, dd, J = 7.9 Hz, J = 6.6 Hz, 3-methine proton), 4.29-4.21 and 4.09-4.01 (2H, 2m, 1-methylene protons), 3.15-3.03 and 2.51-2.39 (2H, 2m, 2- methylene), 2.58 (3H, s, 7-methyl); MS (EI mode) m/z 310 and 312 (M^+ , ^{79}Br & ^{81}Br), 293 and 295 (M^+ , $-\text{NH}_3$), 263 and 265 (M^+ , $-\text{NH}_3$, $-\text{NO}$). Rotations: $\text{R}(-)[\alpha]_{\text{D}}^{25}$ = -8.1° (C = 0.42, MeOH); $\text{S}(+)[\alpha]_{\text{D}}^{25}$ = +10.1° (C = 0.16, MeOH).

Anal. ($\text{C}_{11}\text{H}_{11}\text{N}_4\text{O}_2 \cdot 0.25\text{H}_2\text{O}$) C, H, N.

3-Amino-6-azindinyl-2,3-dihydro-7-methyl-1H-pyrrolo[1,2-*a*]benzimidazole-5,8-dione(2) A solution consisting of 70 mg (0.225 mmol) of **9**, 70 mg of 5% Pd on charcoal, and 70 mL of methanol was shaken under 50 psi H_2 for 24h. The reaction mixture was filtered through Celite, evaporated to dryness, and then combined with a solution of 0.333 g KH_2PO_4 in a 30 mL of water. The resulting solution was combined with another solution consisting of 1.0 g of KH_2PO_4 and 0.700 g Fremy salt in 50 mL of water. The reaction mixture was stirred at room temperature for 6h and then concentrated under high vacuum to a residue, which was placed on a 25 mL Baker Bond Phenyl reverse phase column prepared with 100% water. The unstable aminoquinone was eluted from the column with water and the combined fractions were concentrated to a dry residue, which was dissolved in 10 mL of methanol and 0.3 mL of aziridine. This reaction mixture was stirred at room temperature for 5 h. The solvent was evaporated and the residue was purified by flash column chromatography employing

chloroform/methanol [95:5] as the eluent. The product fractions were evaporated to dryness and the residue recrystallized from chloroform/hexane to afford **2** as a red solid: 6.0 mg (10.3%) overall yield; mp>240°C, dec; TLC (chloroform/methanol [80:20]) R_f = 0.17; IR (KBr pellet) 3387, 2996, 2924, 1674, 1632, 1576, 1518, 1377, 1341, 1312, 1140, 988, cm^{-1} ; ^1H NMR (DMSO- d_6) δ 4.23 - 4.15 and 4.06 - 3.97 (3H, 2m, 3-methine and 1-methylene), 2.89 - 2.78 and 2.27 - 2.16 (2H, 2m, 2- methylene), 2.29 (4H, s, aziridinyl protons), 1.94 (3H, s, 7-methyl); MS (EI mode) m/z 258 (M^+), 240, 214. Anal.($\text{C}_{13}\text{H}_{14}\text{N}_4\text{O}_2 \cdot 0.35\text{H}_2\text{O}$) C, H, N.

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6-Bromo-3-Carbamido-2,3-dihydro-7-methyl-5-nitro-1H-pyrrolo[1,2-a]benzimidazole (10) was prepared by the following two- step procedure starting with **9**.

A solution of 150 mg (0.482 mmol) **9** in 9 mL of dry pyridine was cooled to 0°C in an ice bath. To the cooled solution was added 0.3 mL of phenylchloroformate and the reaction mixture was stirred at 0°C for 15 min. The mixture was then removed from the ice bath and allowed to warm to room temperature with stirring over 1h. The reaction mixture was diluted with 50 mL of ethyl acetate and the resulting mixture washed 3x with 15 mL of 20% aqueous acetic acid, 2x with 10 mL portions of 0.12N aqueous hydrochloric acid, and finally

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2x with 15 mL of water. The ethyl acetate layer was dried over Na_2SO_4 and the solvent was evaporated to a residue, which was recrystallized from chloroform/hexane to afford the phenoxyamido derivative as a light brown solid: 162 mg (78%) yield; mp 175-178 °C; TLC (chloroform/methanol [80:20]) R_f = 0.66; IR (KBr pellet) 3306, 1736, 1537, 1491, 1208 cm^{-1} ; ^1H NMR (DMSO- d_6) δ 8.55 (1H, d, J =9 Hz, amide proton), 7.87 (1H, s, C(8) proton), 7.4-6.7 (5H, 3m, aromatic protons), 5.28 (1H, q, J = 6.6 Hz, 3-methine), 4.36-4.26 and 4.18-4.09 (2H, 2m, 1- methylene), 3.13-3.01 and 2.61-2.49 (2H, 2m, 2-methylene), 2.54 (3H, s, 7-methyl); MS (EI mode) m/z 430 and 432 (M^+ , ^{79}Br & ^{81}Br). Rotations: $R(-)[\alpha]_D^{25} = +13.6^\circ$ (C = 0.26, MeOH); $S(+)[\alpha]_D^{25} = -14.0^\circ$ (C = 0.33, MeOH).

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Anal. ($\text{C}_{18}\text{H}_{15}\text{BrN}_4\text{O}_4 \cdot 0.25 \text{H}_2\text{O}$) C, H, N.

Gaseous ammonia was passed into a dry flask cooled to -70°C with isopropanol/dry ice bath until 20 mL of liquid ammonia was obtained. A solution of 150 mg (0.348 mmol) of the phenoxyamido derivative in 10 mL of dry methylene chloride was added to the liquid ammonia and the resulting mixture was stirred for 30 min at -70°C . The reaction mixture was then removed from the dry ice bath and stirred for 3h at room temperature. The solvent was evaporated and the solid residue was recrystallized from chloroform/hexane to afford **10** as an off-white solid: 104 mg (84.5%) yield; mp $240-241^{\circ}\text{C}$; TLC (chloroform/methanol [80:20]) $R_f = 0.34$; IR (KBr pellet) 3410, 3293, 2922, 1657, 1588, 1532, 1371, cm^{-1} ; ^1H NMR (DMSO d_6) δ 7.83 (1H, d, $J = 1.2$ Hz, aromatic proton), 6.69 (1H, d, $J = 8.4$ Hz, amide protons), 5.69 (2H, s, amide protons), 5.22 (1H, m, 3-methine), 4.30-4.21 and 4.11-4.03 (2H, 2m, 1- methylene), 2.99-2.88 and 2.41-2.30 (2H, 2m, 2- methylene), 2.54 (3H, s, 7-methyl); MS (EI mode) m/z 353 and 355 (M^+ , ^{79}Br & ^{81}Br). Anal($\text{C}_{12}\text{H}_{12}\text{N}_3\text{O}_3 \cdot 0.5\text{H}_2\text{O}$) C, H, N.

3-Carbamido-6-aziridinyl-2,3-dihydro-7-methyl-1H-pyrrolo[1,2-a]benzimidazole-5,8-dione(3) was prepared from **10** by the following two-step procedure. A solution of 120 mg (0.339 mmol) of **10**, 80 mg of 5% Pd on charcoal, and 200 mL of methanol was shaken under 50 psi H for 15h. The reaction mixture was filtered through Celite and the solvent was removed by evaporation. The residue was dissolved into a solution of 296 mg of KH_2PO_4 in 120 mL of water. To this solution was added a Fremy salt solution consisting of 1.2 g of Fremy salt and 1.8 g KH_2PO_4 in 174 mL of water. The reaction was stirred at room temperature for 1.5 h and then concentrated with high vacuum. The residue was placed on a 25 mL Bakerbond Phenyl reverse phase column prepared with 100% water. The yellow product fractions were collected from the column employing water as the eluent. The fractions were evaporated to dryness and the residue recrystallized from ethyl acetate /hexane: 46 mg (52%) yield; mp $180-183^{\circ}\text{C}$ TLC (chloroform/methanol [80:20]) $R_f = 0.48$; IR (KBr pellet) 3393, 3297, 3212, 1659, 1593, 1570, 1516, 1329, 1152 cm^{-1} ; ^1H NMR (DMSO- d_6) δ 6.64 (1H, d, $J = 8.4$ Hz, amide proton), 6.57 (1H, d, $J = 2.1$ Hz, aromatic proton), 5.67 (2H, s, amine protons), 5.09-5.01 (1H, m, 3-methine), 4.29-4.21 and 4.14-4.06 (2H, 2m,

1 methylene), 2.99-2.88 and 2.41-2.30 (2H, 2m, 2 methylene), 2.01 (3H, d, $J = 1.5$ Hz, 7-methyl); MS (EI mode) m/z 260 (M^+), 243 ($M^+ - NH_3$), 217 ($M^+ - H - N=C=O$). Anal($C_{12}H_{12}N_4O_3 \cdot 0.35H_2O$) C, H, N.

A solution of 20 mg (0.077 mmol) of the carbamide quinone, 10 mL of dry methanol, and 0.3 mL of aziridine were stirred at room temperature for 3h. The reaction solvent was evaporated and the red residue was dissolved into a 9:1 chloroform/methanol mixture and purified by flash column chromatography using 9:1 chloroform/methanol as the eluent. Evaporation of the solvent gave a red solid which was recrystallized from chloroform/hexane: 8.1 mg (35.0%) yield; mp 200-205°C; TLC (chloroform/methanol [80:20]) $R_f = 0.37$; IR (KBr pellet) 3351, 1678, 1647, 1522, 1316, cm^{-1} ; 1H NMR (DMSO- d_6) δ 6.62 (1H, d, $J = 7.8$ Hz, amide proton), 5.65 (2H, s, amine protons), 5.04-4.96 (1H, m, 3-methine), 4.25-4.17 and 4.10-4.01 (2H, 2m, 1- methylene), 2.97-2.86 and 2.39-2.30 (2H, 2m, 2- methylene), 2.30 (4H, s, aziridiny protons), 1.95 (3H, s, 7-methyl); MS (EI mode) m/z 301 (M^+). Anal. ($C_{14}H_{15}N_5O_3 \cdot 1.0H_2O$): C, H, N.

- Alkylation of DNA by Reduced PBIs.** To a mixture of 1-2 mg of DNA (poly[dA], poly[dT], poly[dG], poly[dC], poly[dA- dT], poly[dT- dA]) in 2.0 mL of 0.05 M of pH 7.4 tris buffer and 2 mg of Pd on carbon was added a five-to-one base pair equivalent amount of the PBI dissolved in 0.5 mL of dimethylsulfoxide.
- 20 The resulting solution was degassed under argon for 30 min., after which the mixture was purged with H_2 for 10 min. The solution was then purged with argon for 10 min. and the placed in a 30° C bath for 24 h. The reaction was opened to the air and the catalyst was removed with a Millex-PF 0.8 μ M syringe filter. The filtrate was adjusted to 0.3M acetate with a 3M stock solution of pH 5.1 acetate and the diluted with two volumes of ethanol. The mixture was chilled at -20°C for 12 h and the DNA pellet collected by centrifuging at 12,000 g for 20 min. The pellet was redissolved in water and then precipitated and centrifuged again. The resulting pellet was suspended in ethanol, centrifuged, and dried.

- DT-Diaphorase Reduction Kinetics Studies.** Kinetic studies were
- 30 carried out in 0.05 M pH 7.4 tris.HCl buffer, under anaerobic conditions, employing Thunberg cuvettes. A 2 mM stock solution of the appropriate PBI or

- APBI was prepared in dimethyl sulfoxide (DMSO). To the top port was added the quinone stock and to the bottom port was added DT-diaphorase and NADH in the tris buffer. The top and bottom ports were purged with argon for 20 minutes and equilibrated to 30°C. The ports were then mixed and the reaction followed at 296 nm for 25 minutes to obtain initial rates. The concentrations upon after mixing were 0.3 mM NADH, 1 to 20 x 10⁻⁵ M quinone, and 14.5 nM (based on flavin) of enzyme active sites. The value of $\Delta\epsilon$ was calculated from the initial and final absorbance values for complete quinone reduction, usual value for $\Delta\epsilon$ is 6000 to 8000 M⁻¹ cm⁻¹. The value of $\Delta\epsilon$ was used to calculate V_{max} in M sec⁻¹. The results were fitted to a Lineweaver-Burke plot from which k_{cat}/K_m values were calculated based on 14.5 nM of active sites.

Table 1. Percentages of PBI Incorporation into Various DNAs

DNA	S(-)-A	R(+)-A	S(-)-B	R(+)-B	S(-)-C	R(+)-C
Poly [dG]						
Poly [dC]	6.6	9.9	52	105	12.4	17
Poly [dA]						
Poly [dT]	17.4	101	186	60	70	31

Table 2. Parameters for DT-diaphorase Substrate Activity and Melanoma an Ovarian Cancer Log₁₀LC₅₀ (Moles/liter) Values for APBI Analogues

PBI	$K_m \times 10^5$	V_{max} (nM sec ⁻¹)	$K_{cat}/K_m \times 10^4$
S(-)-A	12.7	47.6	3.74
R(+)-A	3.8	28	7.5
S(-)-B			
R(+)-B	2.4	14.6	6.1
S(-)-C	5.15	21.8	4.23
R(+)-C	4.6	39.3	8.59

Compound #	Formula	Calculated			Found		
		C %	H %	N %	C %	H %	N %
5	1	1	1	1	1	1	1
6	2	2	2	2	2	2	2
7	3	3	3	3	3	3	3
8	4	4	4	4	4	4	4
9	5	5	5	5	5	5	5
10	6	6	6	6	6	6	6
11	7	7	7	7	7	7	7
12	8	8	8	8	8	8	8
13	9	9	9	9	9	9	9
14	10	10	10	10	10	10	10
15	11	11	11	11	11	11	11
16	12	12	12	12	12	12	12
17	13	13	13	13	13	13	13
18	14	14	14	14	14	14	14
19	15	15	15	15	15	15	15
20	16	16	16	16	16	16	16
21	17	17	17	17	17	17	17
22	18	18	18	18	18	18	18
23	19	19	19	19	19	19	19
24	20	20	20	20	20	20	20
25	21	21	21	21	21	21	21
26	22	22	22	22	22	22	22
27	23	23	23	23	23	23	23
28	24	24	24	24	24	24	24
29	25	25	25	25	25	25	25
30	26	26	26	26	26	26	26
31	27	27	27	27	27	27	27
32	28	28	28	28	28	28	28
33	29	29	29	29	29	29	29
34	30	30	30	30	30	30	30
35	31	31	31	31	31	31	31
36	32	32	32	32	32	32	32
37	33	33	33	33	33	33	33
38	34	34	34	34	34	34	34
39	35	35	35	35	35	35	35
40	36	36	36	36	36	36	36
41	37	37	37	37	37	37	37
42	38	38	38	38	38	38	38
43	39	39	39	39	39	39	39
44	40	40	40	40	40	40	40
45	41	41	41	41	41	41	41
46	42	42	42	42	42	42	42
47	43	43	<				

The dosage administered will be dependent upon the identity of the neoplastic disease; the types of host involved, including its age, health and weight; the kind of concurrent treatment, if any; the frequency of treatment and therapeutic ratio.

intravenous, 0.1 to about 200 mg/kg; intramuscular, 1 to about 500 mg/kg; orally, 5 to about 1000 mg/kg; intranasal instillation, 5 to about 1000 mg/kg; and aerosol, 5 to about 1000 mg/kg of host body weight.

SUBSTITUTE SHEET (RULE 26)

The compositions of the present invention are preferably presented for administration to humans and animals in unit dosage forms, such as tablets, capsules, pills, powders, granules, suppositories, sterile parenteral solutions or suspensions, sterile non-parenteral solutions of suspensions, and oral solutions or suspensions and the like, containing suitable quantities of an active ingredient.

For oral administration either solid or fluid unit dosage forms can be prepared.

Powders are prepared quite simply by comminuting the active ingredient to a suitably fine size and mixing with a similarly comminuted diluent. The diluent can be an eatable carbohydrate material, such as lactose or starch, advantageously, a sweetening agent or sugar is present as well as a flavoring oil.

Capsules are produced by preparing a powder mixture as hereinbefore described and filling into formed gelatin sheaths. Advantageously, as an adjuvant to the filling operation, a lubricant such as talc, magnesium stearate, calcium stearate and the like is added to the powder mixture before the filling operation.

Soft gelatin capsules are prepared by machine encapsulation of a slurry of active ingredients with an acceptable vegetable oil, light liquid petrolatum or other inert oil or triglyceride.

Tablets are made by preparing a powder mixture, granulating or slugging, adding a lubricant and pressing into tablets. The powder mixture is prepared by mixing an active ingredient, suitably comminuted, with a diluent or base such as starch, lactose, kaolin, dicalcium phosphate and the like. The powder mixture can be granulated by wetting with a binder such as corn syrup, gelatin solution, methylcellulose solution or acacia mucilage and forcing through a screen. As an alternative to granulating, the powder mixture can be slugged, i.e., run through the tablet machine and the resulting imperfectly formed tablets broken into pieces (slugs). The slugs can be lubricated to prevent sticking to the tablet-forming dies by means of the addition of stearic acid, a stearic salt, talc or mineral oil. The lubricated mixture is then compressed into tablets.

Advantageously, the tablet can be provided with a protective coating consisting of a sealing coat or enteric coat of shellac, a coating of sugar and methylcellulose and polish coating of carnauba wax.

Fluid unit dosage forms for oil administration such as in syrups, elixirs and suspensions can be prepared wherein each teaspoonful of composition contains a predetermined amount of an active ingredient for administration. The water-soluble forms can be dissolved in an aqueous vehicle together with sugar, flavoring agents and preservatives to form a syrup. An elixir is prepared by using a hydroalcoholic vehicle with suitable sweeteners together with a flavoring agent. Suspensions can
10 be prepared of the insoluble forms with a suitable vehicle with the aid of a suspending agent such as acacia, tragacanth, methylcellulose and the like.

For parenteral administration, fluid unit dosage forms are prepared utilizing an active ingredient and a sterile vehicle, water being preferred. The active ingredient, depending on the form and concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the water-soluble active ingredient can be dissolved in water for injection and filter sterilized before filling into a suitable vial or ampule and sealing. Advantageously, adjuvants such as a local anesthetic, preservative and buffering agents can be dissolved in the vehicle. Parenteral suspensions are prepared in substantially the same manner except that an
20 active ingredient is suspended in the vehicle instead of being dissolved and sterilization cannot be accomplished by filtration. The active ingredient can be sterilized by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the active ingredient.

In addition to oral and parenteral administration, the rectal and vaginal routes can be utilized. An active ingredient can be administered by means of a suppository. A vehicle which has a melting point at about body temperature or one that is readily soluble can be utilized. For example, cocoa butter and various polyethylene glycols (Carbowaxes) can serve as the vehicle.

For intranasal instillation, a fluid unit dosage form is prepared utilizing an active ingredient and a suitable pharmaceutical vehicle, preferably P.F. water, a dry powder can be formulated when insufflation is the administration of choice.

For use as aerosols, the active ingredients can be packaged in a pressurized aerosol container together with a gaseous or liquified propellant, for example, dichlorodifluoromethane, carbon dioxide, nitrogen, propane, and the like, with the usual adjuvants such as cosolvents and wetting agents, as may be necessary or desirable.

10 The term "unit dosage form" as used in the specification and claims refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical diluent, carrier or vehicle. The specifications for the novel unit dosage forms of this invention are dictated by and are directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitation inherent in the art of compounding such an active material for therapeutic use in humans, as disclosed in this specification, these being features of the present invention. Examples of suitable unit dosage forms in accord with this invention are tablets, capsules, troches, suppositories, powder packets, 20 wafers, cachets, teaspoonfuls, tablespoonfuls, dropperfuls, ampules, vials, segregated multiples of any of the foregoing, and other forms as herein described.

The active ingredients to be employed as antineoplastic agents can be easily prepared in such unit dosage form with the employment of pharmaceutical materials which themselves are available in the art and can be prepared by established procedures. The following preparations are illustrative of the preparation of the unit dosage forms of the present invention, and not as a limitation thereof. Several dosage forms were prepared embodying the present invention. They are shown in the following examples in which the notation "active ingredient" signifies either cyclopent(b)indole 3b-e and/or indole 2a-c, and/or indole 1a-c or any other 30 compound described herein.

COMPOSITION "A"

Hard-Gelatin Capsules

One thousand two-piece hard gelatin capsules for oral use, each capsule containing 200 mg of an active ingredient are prepared from the following types and amounts of ingredients:

Active ingredient, micronized	200 g
Corn Starch	20 g
Talc	20 g
Magnesium stearate	2 g

Active ingredient, finely divided by means of an air micronizer, is added to the other finely powdered ingredients, mixed thoroughly and then encapsulated in the usual manner.

The foregoing capsules are useful for treating a neoplastic disease by the oral administration of one or two capsules one to four times a day.

- 10 Using the procedure above, capsules are similarly prepared containing an active ingredient in 50, 250 and 500 mg amounts by substituting 50 g, 250 g and 500 g of an active ingredient for the 200 g used above.

COMPOSITION "B"

Soft Gelatin Capsules

One-piece soft gelatin capsules for oral use, each containing 200 mg of an active ingredient, finely divided by means of an air micronizer, are prepared by first suspending the compound in 0.5 ml of corn oil to render the material capsulatable and then encapsulating in the above manner.

- 20 The foregoing capsules are useful for treating a neoplastic disease by the oral administration of one or two capsules one to four times a day.

COMPOSITION "C"

Tablets

One thousand tablets, each containing 200 mg of an active ingredient are prepared from the following types and amounts of ingredients:

Active ingredient, micronized	200 g
Lactose	300 g

25

Corn starch	50 g
Magnesium stearate	4 g
Light liquid petrolatum	5 g

The active ingredient, finely divided by means of an air micronizer, is added to the other ingredients and then thoroughly mixed and slugged. The slugs are broken down by forcing them through a Number Sixteen screen. The resulting granules are then compressed into tablets, each tablet containing 200 mg of the active ingredient.

The foregoing tablets are useful for treating a neoplastic disease by the oral administration of one or two tablets one to four times a day.

Using the procedure above, tablets are similarly prepared containing an active ingredient in 250 mg and 100 mg amounts by substituting 250 g and 100 g of an active ingredient for the 200 g used above.

COMPOSITION "D"

Oral Suspension

One liter of an aqueous suspension for oral use, containing in each teaspoonful (5 ml) dose, 50 mg of an active ingredient, is prepared from the following types and amounts of ingredients.

Active ingredient, micronized	10 g
Citric acid	2 g
Benzoic acid	1 g
Sucrose	790 g
Tragacanth	5 g
Lemon Oil	2 g
Deionized water, q.s.	1000 ml

The citric acid, benzoic acid, sucrose, tragacanth and lemon oil are dispersed in sufficient water to make 850 ml of suspension. The active ingredient, finely divided by means of an air micronizer, is stirred into the syrup unit uniformly distributed. Sufficient water is added to make 1000 ml.

The composition so prepared is useful for treating a neoplastic disease at a dose of 1 teaspoonful (15 ml) three times a day.

COMPOSITION "E"**Parenteral Product**

The sterile aqueous suspension for parenteral injection, containing 30 mg of an active ingredient in each milliliter for treating a neoplastic disease, is prepared from the following types and amounts of ingredients:

Active ingredient, micronized	30 g
POLYSORBATE 80	5 g
Methylparaben	2.5 g
Propylparaben	0.17 g
Water for injection, q. s.	1000 ml.

All ingredients, except the active ingredient, are dissolved in the water and the solution sterilized by filtration. To the sterile solution is added the sterilized active ingredient, finely divided by means of an air micronizer, and the final suspension is filled into sterile vials and the vials sealed.

- 10 The composition so prepared is useful for treating a neoplastic disease at a dose of 1 milliliter (1 ml) three times a day.

COMPOSITION "F"**Suppository, Rectal and Vaginal**

One thousand suppositories, each weighing 2.5 g and containing 200 mg of an active ingredient are prepared from the following types and amounts of ingredients:

Active ingredient, micronized	15 g
Propylene glycol	150 g
Polyethylene glycol #4000, q. s.	2,500 g

- 20 The active ingredient is finely divided by means of an air micronizer and added to the propylene glycol and the mixture passed through a colloid mill until uniformly dispersed. The polyethylene glycol is melted and the propylene glycol dispersion is added slowly with stirring. The suspension is poured into unchilled molds at 40°C. The composition is allowed to cool and solidify and then removed from the mold and each suppository foil wrapped.

The foregoing suppositories are inserted rectally or vaginally for treating a neoplastic disease.

COMPOSITION "G"

Intranasal Suspension

Active ingredient, micronized	15 g
POLYSORBATE 80	5 g
Methylparaben	2.5 g
Propylparaben	0.17 g
Deionized water, q.s.	1000 ml.

All ingredients, except the active ingredient, are dissolved in the water and the solution sterilized by filtration. To the sterile solution is added the sterilized active ingredient, finely divided by means of an air micronizer, and the final suspension is aseptically filled into sterile containers.

- The composition so prepared is useful for treating a neoplastic disease, by
- 10 intranasal instillation of 0.2 to 0.5 ml given one to four times per day.

An active ingredient can also be present in the undiluted pure form for use locally about the cutis, intranasally pharyngolaryngeally, bronchially, or orally.

COMPOSITION "H"

Powder

Five grams of an active ingredient in bulk form is finely divided by means of an air micronizer. The micronized powder is placed in a shaker-type container.

The foregoing composition is useful for treating a neoplastic disease, at localized sites by applying a powder one to four times per day

COMPOSITION "I"

20

Oral Powder

One hundred grams of an active ingredient in bulk form is finely divided by means of an air micronizer. The micronized powder is divided into individual doses of 200 mg and packaged.

The foregoing powders are useful for treating a neoplastic disease, by the oral administration of one or two powders suspended in a glass of water, one to four times per day.

COMPOSITION "J"

Insufflation

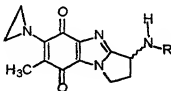
One hundred grams of an active ingredient in bulk form is finely divided by means of an air micronizer.

The foregoing composition is useful for treating antineoplastic disease, by the inhalation of 300 mg one to four times a day.

From the foregoing, it becomes readily apparent that a new and useful antineoplastic factor and new and useful antineoplastic preparations have been herein described and illustrated which fulfill all of the aforestated objectives in a remarkably unexpected fashion. It is of course understood that such modifications, alterations and adaptations as will readily occur to the artisan confronted with this disclosure are intended within the spirit of the present invention.

Claims:

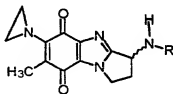
1. A pharmaceutical preparation for treating neoplastic disease comprising a pharmaceutically acceptable carrier and a tumor-inhibiting amount of a yujungamycin.
2. A preparation according to claim 1 in which said yujungamycins have the structural formula:



wherein: R = H, CONH₂, COCH₃.

3. A pharmaceutical preparation according to claim 2 in which said yujungamycins has a configuration of S(-) or R(+).

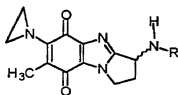
4. A pharmaceutical preparation according to claim 1 in which said yujungamycin is selected from the group consisting of 3-acetamido-6-aziridinyl-2,3-dihydro-7-methyl-1*H*-pyrrolo[1,2- α] benzimidazole-5,8-dione; 3-amino-6-aziridinyl-2,3-dihydro-7-methyl-1*H*-pyrrolo[1,2- α] benzimidazole-5,8-dione; and 3-carbamido-6-aziridinyl-2,3-dihydro-7-methyl-1*H*-pyrrolo[1,2- α] benzimidazole-5,8-dione.
5. A pharmaceutical preparation for treating a neoplastic disease selected from the group consisting of central nervous system cancer, colon cancer, ovarian cancer, and lung cancer comprising a pharmaceutically acceptable carrier and a tumor-inhibiting amount of a compound selected from the group consisting of yujungamycins having the structural formula:



wherein R is selected from the group consisting of H, COCH₃, and CONH₂.

6. A pharmaceutical preparation according to claim 5 in which said yujungamycins has a configuration of S(-) or R(+).
7. A pharmaceutical preparation according to claim 5 in which said yujungamycin is selected from the group consisting of 3-acetamido-6-aziridinyl-2,3-dihydro-7-methyl-1*H*-pyrrolo[1,2- α] benzimidazole-5,8-dione; 3-amino-6-aziridinyl-2,3-dihydro-7-methyl-1*H*-pyrrolo[1,2- α] benzimidazole-5,8-dione; and 3-carbamido-6-aziridinyl-2,3-dihydro-7-methyl-1*H*-pyrrolo[1,2- α] benzimidazole-5,8-dione.

8. A method of treating a host afflicted with a neoplastic disease selected from the group consisting of central nervous system cancer, colon cancer, ovarian cancer, and lung cancer comprising administering to said host an effective amount of a compound selected from the group consisting of yujungamycins having the structural formula



wherein: R is H, CONH₂ or COCH₃.

9. A pharmaceutical preparation according to claim 8 in which said yujungamycins has a configuration of S(-) or R(+).
10. A pharmaceutical preparation according to claim 8 in which said yujungamycin is selected from the group consisting of 3-acetamido-6-aziridinyl-2,3-dihydro-7-methyl-1*H*-pyrrolo[1,2- α] benzimidazole-5,8-dione; 3-amino-6-aziridinyl-2,3-dihydro-7-methyl-1*H*-pyrrolo[1,2- α] benzimidazole-5,8-dione; and 3-carbamido-6-aziridinyl-2,3-dihydro-7-methyl-1*H*-pyrrolo[1,2- α] benzimidazole-5,8-dione.

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Assignee of record of the entire interest.

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Attorney or Agent of record.



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Name

RICHARD R. MYBECK

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Richard R Mybeck

Date

18 July 2001

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DECLARATION AND POWER OF ATTORNEY

Docket 5452

I, the below named inventor, hereby declare that:

My residence, post office addresses and citizenship is stated below next to my name.

I believe that I am the original, first and sole inventor of the subject matter which is described in the Application for the invention entitled: TREATMENT OF NEOPLASMS WITH THE YUJUNGAMYCINS, the Specification of which is attached hereto.

I hereby state that I have reviewed and understand the contents of the above-identified specification.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, 1.56(a).

I do not know and do not believe the same was ever known or used in the United States of America before our invention thereof, or patented or described in any printed publication in any country before our invention thereof or more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by us or our legal representatives or assigns more than twelve months prior to this application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby request that all correspondence be directed to RICHARD R. MYBECK, Post Office Box 5540, Scottsdale, Arizona 85258-1234 and that all telephone calls be directed to RICHARD R. MYBECK at telephone no. (480)483-1285.

[illegible]

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Date: 7/18/2001